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THE EFFECTS OF DESALIVATION BY DUCT LIGATION OR SALIVARY GLAND EXTIRPATION ON TASTE PREFERENCE IN RATS

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Summary—Albino rats were desalivated by either duct ligation or extirpation of the major salivary glands. The preference tests consisted of a choice between two cups containing a solid diet or between two bottles containing solutions. The flavour additives were quinine sulphate or NaCl. Rats normally have a slight preference for salt diets and show a clear aversion for bitter tastes. Short-term testing of the desalivated rats started 4 days after surgery and long-term testing at 104 days. Each test group consisted of 6–9 rats and the same number of controls. The proportion of flavoured food or fluid selected relative to total intake was analysed statistically. Both duct-ligated and gland-extirpated rats were hypogeusic to quinine- and NaCl-containing solutions. Neither the mode of desalivation nor the time factor played a role in the acceptance of the quinine- or NaCl-flavoured solutions. Presentation of the same taste stimuli in either a liquid or solid medium resulted in different taste response of the desalivated rats. The texture qualities of the diet were of greater importance in acceptance of food than taste *per se*.

INTRODUCTION

Surgical desalivation produces changes in taste preference in rats which Vance (1965), Kissileff (1967) and Catalanotto and Sweeney (1972) attributed to altered taste sensitivity. However, Brill and Maller (1972) and Lawson, Hagstrom and Walter (1974) were unable to corroborate these findings. This disparity may be due to differences of method, such as the surgical desalivatory techniques, testing procedures and time intervals between surgery and preference testing.

Vance (1965) reported marked enhancement of preference for NaCl solutions immediately after duct ligation which disappeared 30 days later. Catalanotto and Sweeney (1972) found high preference for NaCl solutions in both acutely (6 days) and chronically (7 months post-surgery) gland-extirpated rats. However, in subsequent experiments, they (Catalanotto and Sweeney, 1973) found that 6 months after extirpation there was a lower preference for NaCl than in the controls and also a loss of aversion to quinine sulphate. Galili, Maller and Brightman (1978) found that pharmacologically-induced xerostomia in rats was accompanied by increased acceptance of NaCl- and citric acid-flavoured solid food. Our aim was to discover more about the time intervals between desalivation and changes in taste function and to compare the effects of duct ligation with those of gland extirpation.

MATERIALS AND METHODS

Surgical procedures

Male Charles River (CD) random-bred albino rats were desalivated surgically by either duct ligation or extirpation of the major salivary glands. The parotid ducts were ligated at the site where they cross the lateral aspect of the masseter muscles. The submandibular and the major sublingual ducts were ligated prior to their disappearance beneath the anterior bellies of the digastric muscles. Two ligatures 1–2 mm apart, were placed on each duct (Vance, 1965). The rats were anaesthetized with sodium pentobarbital, 36 mg/kg intraperitoneally (i.p.).

Maintenance of animals

The rats were housed in individual wire-mesh cages in a temperature-constant room (23–26°C) with a reversed light–dark cycle (8 h dark, 16 h light). They were maintained *ad libitum* on powdered diet (Purina rat meal) and water. Five per cent (w/w) of jelly petrolatum (Vaseline) was added to all the powdered diets (except in the texture experiment) to facilitate eating by the xerostomic rats; food consumption and body weight decreased dramatically following surgery. The flavour additives and concentrations were as follows: NaCl at 0.025, 0.25, 0.5 or 1.0 per cent (w/w) for the powdered diet, and concentrations of 2.5×10^{-2}, 5×10^{-2} or 1×10^{-1} M dissolved in deionized water; quinine sulphate at 1.3×10^{-6}, 1.3×10^{-5} or 3.3×10^{-5} M dissolved in deionized water; sucrose octaacetate (2 per cent w/w) was added to the powdered diet in the texture experiment.

The concentrations for the solutions were selected on the basis of information obtained from the experiments of Vance (1965) and Catalanotto and Sweeney (1972, 1973).

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Solid-food preference test shortly after duct ligation

Six rats with duct ligation were compared with 6 controls of similar weight (500 g). Testing began 4 days after ligation. Each animal was given a choice of two identical cups, one containing powdered diet with NaCl and the other without. The 1 per cent NaCl diet was offered for 48 h, the position of the cups being reversed after 24 h. The unflavoured diet alone was then offered for 48 h, followed by the choice of the next lower concentration of NaCl for 48 h, and so on down to the 0.025 per cent NaCl level. The alternation reduced any carry-over effect of preference from previous stimuli. Food and water consumption was measured at 4 and 24 h after onset of the 8 h dark period to allow for differences in short- and long-term preference (Cagan and Maller, 1974). Water intake was measured in a commercially available glass device graduated in 1 ml increments; food intake was measured by weighing the food cup before and after the test period and carefully collecting spilled food from beneath the cages. The rats in this experiment had been used previously in a study in which xerostomia was induced pharmacologically by scopolamine methyl nitrate (Galili *et al.*, 1978). Ten days elapsed between the last drug administration and surgery.

Two-bottle solution-preference test shortly after duct ligation

Seven rats with ligated duct were compared with 7 control rats of similar weight (525 g) in a 24 h two-bottle preference test (Kare and Ficken, 1963). The choice was between quinine sulphate or NaCl solution and deionized water. Testing with the quinine solution began 4 days and with NaCl 16 days after ligation. The solutions were placed in commercial glass drinking vessels with 1 mm graduations. The choice was presented for 48 h, with a change of solutions at 24 h. Quinine sulphate solution was presented first and the three concentrations were given in ascending order. Between each 48 h test period the animals had 48 h with deionized water only.

Two-bottle solution-preference test 3 months after duct ligation

Nine rats with ligated duct were compared with 8 control rats. Six experimental and 6 control rats had been previously studied immediately after ligation. The control rats were heavier (590 g compared with 460 g). Preference tests as above were begun 109 and 122 days after ligation.

Two-bottle solution-preference test 3 months after salivary gland extirpation

Six rats with salivary gland extirpation were compared with 6 control rats which were heavier (490 g versus 250 g). The first test with quinine was at 101 days and with NaCl at 113 days after extirpation. Weight loss on a dry diet was so great that, after 56 days, 5 per cent Vaseline was added to the diet.

Texture-preference test 4 months after salivary-gland extirpation

The six rats with salivary-gland extirpation were given a texture preference test 1 month after the solution preference test and compared with 6 control animals which were heavier (525 g versus 285 g). Tests were begun at 129 days after gland extirpation. The same procedures as in the solid-food preference test were used, but the choices were: dry diet versus diet with 5 per cent Vaseline, dry diet versus diet with 25 per cent Vaseline, diet with 5 per cent Vaseline versus diet with 25 per cent Vaseline, dry diet with 0.5 per cent sodium saccharin versus diet with 25 per cent Vaseline, dry diet versus diet with 25 per cent Vaseline and 2 per cent sucrose octaacetate (bitter in taste).

Controls

Different, normal animals were used as controls in each experiment.

Assessment of degree of desalivation

After the above tests, the degree of desalivation was assessed in sodium pentobarbital-anaesthetized animals by measuring salivary flow over 15 min in response to pilocarpine HCl 6 mg/kg i.p. (Bernarde *et al.*, 1956; Sweeney *et al.*, 1962). This technique is easy to perform and permits the identification of unsuccessful desalivation, thus eliminating this source of error.

Analysis of data

Food and fluid intake were expressed as proportions to avoid the problem of differing body weights. Equal consumption (proportion of 0.5) was regarded as indifference, variations in consumption were defined as preference or rejection. The data were examined by analysis of variance for repeated measures (Weiner, 1962).

RESULTS

Solid-food preference test shortly after duct ligation

There were no statistically significant differences between the ligated rats and the control animals in their acceptance of salt-flavoured diets at either 4 or 24 h after the presentation of the choice of diets (Fig. 1). However, the ligated animals tended to accept a slightly greater proportion of the salt-flavoured diets. At the first test (4 days after ligation), an increase in

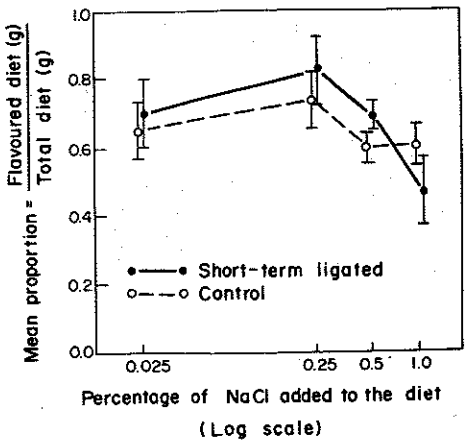

Fig. 1. Mean (±SE) intake of dry diet containing NaCl for 6 short-term ligated rats and 6 controls.

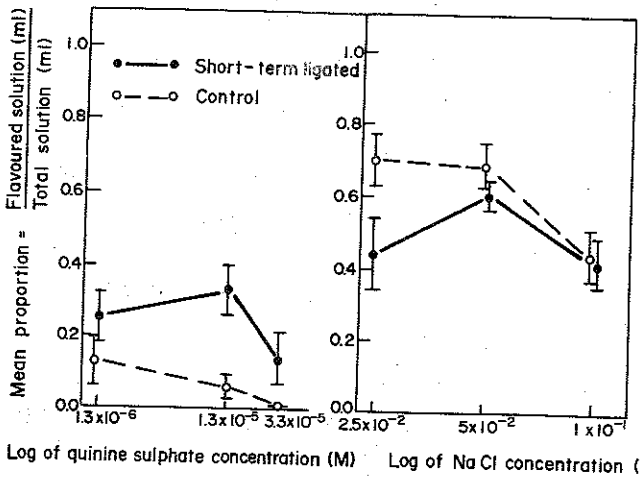

Fig. 2. Mean (±SE) intake of quinine sulphate and NaCl solutions for 7 short-term ligated rats and 7 controls.

water intake of over 30 per cent was noted. Thirty days after ligation, the rats consumed 80 per cent more water than the controls. Vance (1965) also observed polydipsia in desalivated rats, which was explained by Stricker (1970) as being due to prandial drinking. Food intake, on the other hand, did not differ significantly in ligated and control rats.

Two-bottle solution-preference test shortly after duct ligation

Ligated rats displayed an overall higher acceptance of the quinine solutions ($F_{(1,36)} = 13.38$; $p < 0.01$), whereas the control rats showed a marked aversion to the two higher concentrations of quinine sulphate (Fig. 2). The mean proportion intake by the control rats was 6 per cent and 1 per cent, respectively; the ligated rats ingested 33 per cent and 14 per cent of these solutions, respectively.

Acceptance of NaCl solutions was significantly higher by the control rats than by the ligated animals ($F_{(1,36)} = 4.05$; $p < 0.05$). No difference was noted in the mean proportion of liquid intake at the highest NaCl concentration between the two groups: 44 per cent and 43 per cent, respectively. Water intake increased 8 days post-ligation, and continued to increase throughout the rest of the test period, the ligated rats consuming about twice as much water as the control animals.

Two-bottle preference test 3 months after duct ligation

The ligated rats showed an overall higher acceptance of the quinine solutions ($F_{(1,45)} = 31.23$; $p < 0.001$) (Fig. 3). The two highest concentrations were rejected by the control rats, whereas the ligated rats were indifferent towards the highest concentration of the bitter solution (mean proportion intake was 48 per cent and 44 per cent, respectively).

The control rats showed an overall significantly higher acceptance of the saline solutions ($F_{(1,45)} = 8.83$; $p < 0.01$); the duct-ligated rats were indifferent to these taste substances (mean proportion intake of 5×10^{-2} M NaCl was 53 per cent by the xerostomic rats versus 77 per cent by the controls).

Two-bottle solution-preference test 3 months after salivary-gland extirpation

The extirpated rats showed an overall significantly higher acceptance of the quinine solutions

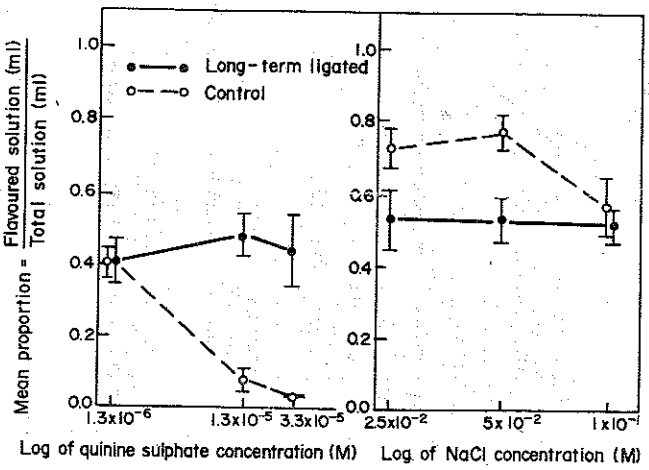

Fig. 3. Mean (±SE) intake of quinine sulphate and NaCl solutions for 9 long-term ligated rats and 8 controls.

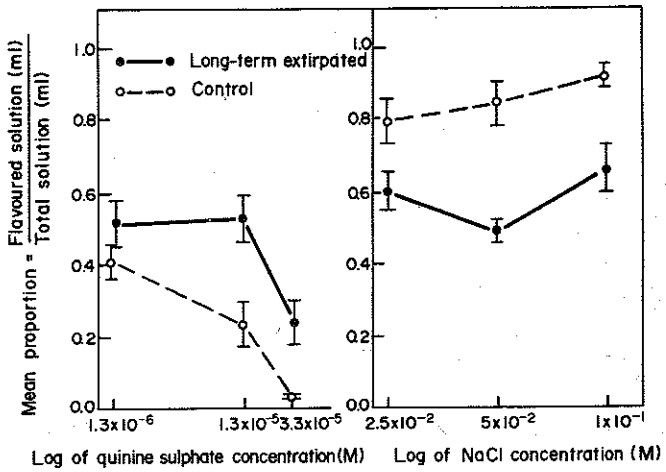

Fig. 4. Mean (±SE) intake of quinine sulphate and NaCl solutions for 6 long-term extirpated rats and 6 controls.

($F_{(1,30)} = 21.54$; $p < 0.01$) (Fig. 4). Whereas the control animals dramatically rejected the highest (3×10^5 M) quinine concentration (mean proportion intake 3 per cent), the extirpated rats accepted it more willingly (mean proportion intake 24 per cent).

There was an overall significantly higher acceptance of the NaCl solutions by the control animals ($F_{(1,30)} = 41.61$; $p < 0.01$), which was expressed as a preference for all three NaCl concentrations (mean proportion intake was 79, 84 and 92 per cent, respectively). The extirpated rats were quite indifferent in their preference for salt solutions.

Texture-preference test 4 months after salivary-gland extirpation

The extirpated rats preferred all five oily diets when they were presented with a choice of oily and dry food (Fig. 5). They not only chose the oily food versus a sweet-flavoured (sodium saccharin) dry diet, but even preferred an oily bitter-flavoured diet to a dry diet. The control rats generally responded indifferently to the possible choices, except for the 5 per cent Vaseline versus the dry-diet choice, where they showed a slightly higher preference for the lubricated food (mean proportion intake 68 per cent). An interesting observation was the lack of rejection by the control group of the 25 per cent Vaseline-lubricated diet containing 2 per cent sucrose octaacetate (mean proportion intake 56 per cent).

The following three preference tests were analysed: a choice between dry and 5 per cent Vaseline-lubricated diets; a choice between dry and 25 per cent Vaseline-lubricated diets; a choice between 5 per cent Vaseline and 25 per cent Vaseline-lubricated diets. The analyses revealed an overall significantly higher acceptance of the oily diets by the extirpated rats ($F_{(1,14)} = 45.58$; $p < 0.01$).

Correlation tests showed that the xerostomic rats preferred the oily diet to the dry sweet food ($t(10) = 4.67$; $p < 0.01$) and more willingly accepted the oily, bitter-flavoured diet than did the control animals (t (10) = 3.59; $p < 0.01$).

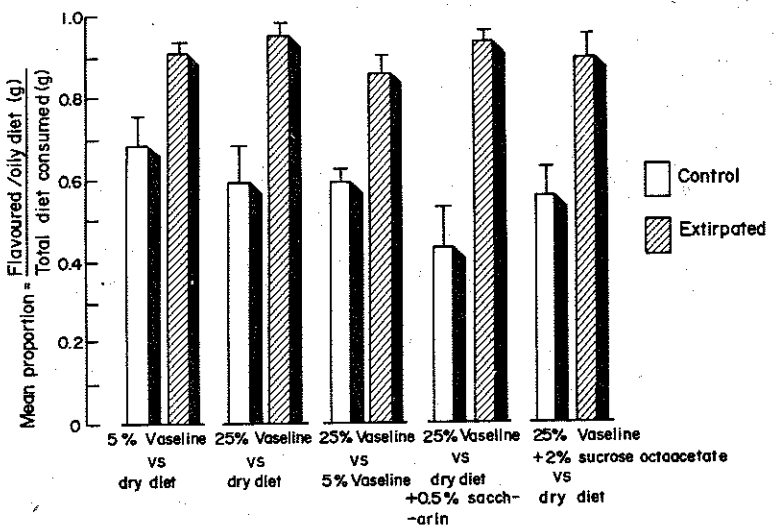

Fig. 5. Preferences for oil and flavour additives in the diet for 6 long-term extirpated rats and 6 controls.

Table 1. Volume of saliva collected from rats desalivated by different surgical techniques

Surgical technique	Post desalivation (days)	Mean body weight (g ± SEM)		Mean saliva volume (ml ± SEM)		No. of rats providing measurable amounts of saliva	
		Experimental	Control	Experimental	Control	Experimental	Control
Ligation	32	429 ± 11.9 (n = 6)	535 ± 9.4 (n = 6)	0.1 ± 0.07	1.75 ± 0.11	2	6
Ligation	132	487 ± 17.4 (n = 9)	625 ± 15.8 (n = 8)	0.06 ± 0.04	1.03 ± 0.06	2	8
Extirpation	144	313 ± 15.5 (n = 6)	531 ± 11.5 (n = 6)	0	1.97 ± 0.11	0	6

Assessment of desalivation

Extirpation proved a more reliable technique than ligation to produce desalivation (Table 1).

DISCUSSION

The results show that there were no differences between acutely or chronically ligated rats in selection of the tested tastants, indicating that post-operative effects of either duct ligation or gland extirpation do not include changes in taste preference. Thus, both the duct-ligated and gland-extirpated rats exhibited pronounced hypogeusia to the NaCl solutions. In general, rats desalivated by either method showed lower acceptance of the NaCl solutions than did their controls. Untreated rats normally prefer saline. However, the desalivated rats exhibited a higher acceptance of quinine solutions than did the control rats, which extensively avoided these bitter-tasting liquids. These results are in keeping with the findings of Catalanotto and Sweeney (1972). However, for salt solutions, our findings contradict those of Vance (1965) and of Catalanotto and Sweeney (1972) who found that ligated and extirpated rats consumed more NaCl solution than did their controls. We observed indifference to salt solutions in the all desalivated animals. These divergent results may be because in the above-mentioned studies the rats were fed a dry diet and prandial drinking was prominent. Catalanotto and Sweeney (1973) used an oily diet containing 28.6 per cent corn oil, which eliminated prandial drinking. Although the diets to which 5 per cent Vaseline was added in our study decreased anorexia and weight loss in the desalivated rats, the diets were not oily enough to eliminate completely the prandial drinking habit.

The consistency of the preferential and aversive behaviour of the three types of desalivated rats (short- and long-term ligated and long-term extirpated animals) in our study is striking. As desalivated rats were relatively indifferent to NaCl solutions and less averse to quinine-containing solutions, a diminution in taste sensitivity is strongly suggested.

Henkin *et al.* (1971, 1972) and Catalanotto and Sweeney (1972) proposed that saliva is involved in the maintenance of normal taste function through its action on the taste-bud epithelium. Nanda and Catalanotto (1978) found mild hyperkeratosis of the tongue epithelium in desalivated rats except in the pore region of the fungiform and vallate papilla. Cano, Roza and Rodriguez-Echandia (1978) and Gomez-Ramos and Rodriguez-Echandia (1979) reported that extirpation of the major salivary glands produced a significant increase in the rate of development of the intermediate type of taste-bud cells in the vallate papilla of the rat 15 days after extirpation. Hence the receptor function of taste-bud cells is sensitive to desalivation. However, because the behavioral changes we observed occurred shortly after desalivation, it is unlikely that they were the consequence of degenerative changes of the taste receptors and epithelium.

Comparison of the responses of surgically desalivated rats to solutions with responses of acute pharmacologically induced-xerostomic rats to flavoured

dry diets (Galili *et al.*, 1978), revealed that the drug-desalivated animals showed a greater acceptance of salt-flavoured diets than did the surgically desalivated ones. The pharmacologically induced xerostomic rats did not differ from control animals in their response to a bitter-flavoured diet (sucrose octaacetate). This discrepancy may be attributed to the different media which contained the tastants (solution versus dry diet), to the different techniques of desalivation and/or to the absence of prandial drinking among the pharmacologically induced xerostomic rats. The rats which had shown a higher acceptance of salt-flavoured diets when desalivated by scopolamine administration than their controls (Galili *et al.*, 1978), did not differ from their controls with regard to salt acceptance after duct ligation. Thus, animals desalivated by varying techniques responded to the same taste stimuli in different ways.

Hamilton (1964) reported that food intake of normal rats may be influenced by the greasiness of the diet. In our texture-preference tests, the behaviour of the xerostomic rats indeed suggested that textural qualities assume greater importance than taste with regard to acceptance of food. They invariably preferred the oily diets, and neither sweetening the dry diet nor adding high amounts of a bitter flavour to the lubricated food altered their preference for the latter.

Changes in salivary functioning effect the perception of both physical and chemical characteristics of the diet, the physical becoming a more powerful stimulus in the control of dietary habits. Dysfunction of salivation produces disorders of oral sensation which include difficulties in taste sensitivity, taste preference and textural preference.

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